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Dust From Pig Confinement Facilities Bind to and Activate Skeletal Muscle Ryanodine Receptor Calcium-Release Channel (RyR1)

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Individuals working in industrial pig farms and residents in communities surrounding these confinement facilities report increased muscle weakness and fatigue. However, causative factors and molecular mechanisms responsible for this remain poorly characterized. In this study we investigated whether dust from confinement facilities contains components that can bind to and activate RyR1. Dust collected 1–2 meters from the ground of pig confinement facilities in Nebraska were extracted with chloroform, filtered and rotor evaporated to dryness. The residues were redissolved in hexane:chloroform (20:1) and precipitates herein referred to as HEX-INS were filtered and air-dried. Thin layer chromatography revealed three major compounds in HEX-INS fractions and ^{1}H NMR suggests these compounds contained fatty acid moieties. In binding assays, HEX-INS displaced [^{3}H]ryanodine from rabbit skeletal muscle RyR1 in a dose-dependent manner, with an IC_{50} of 2.0μg/ml. HEX-INS also displaced [^{3}H]ryanodine from RyR2 (dog heart), but was significantly less potent (IC_{50} of 21.0μg/ml). HEX-INS displacement curves were parallel to that of ryanodine and occurred principally at activating Ca^{2+} concentrations. In single channel studies, HEX-INS increased open probability of RyR1 in a dose-dependent manner (3.5 to 14.0μg/ml, *cis* Ca^{2+} 3μM). At 17.5μg/ml, HEX-INS induced RyR1 in a state of reduced conductance (55.2% of maximum). This subconductance state was more likely to occur and persist at positive holding potentials. Increasing HEX-INS further to 21.0μg/ml resulted in reversible channel closure. At a higher *cis* Ca^{2+} (20μM), lower amounts of HEX-INS were required for induction of the subconductance state and channel closure. These data are the first to demonstrate that pig confinement dust contains component(s) that selectively bind to and activate RyR1, providing a mechanism rationale for reported muscle weakness and fatigue. (Supported in part by grants from NIH to DJR and KRB)

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Ryanodine Receptor Phosphorylation by Protein Kinase a Alters the Affinity for FKBP12

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The Ryanodine Receptor (RyR) ion channel cells plays a vital part in muscle cell Excitation Contraction Coupling by regulating sarcoplasmic reticulum Ca^{2+} release - a process modulated by interaction with FK506 Binding Protein 12 (FKBP12). Pathological hyperphosphorylation of RyR1 by Protein Kinase A (PKA) is reported by some to cause the loss of FKBP12 in excessive exercise and in muscle fatigue reported in heart failure. The physiological relationship between FKBP12 and phosphorylation in channel regulation is uncertain. We have shown previously, using Surface Plasmon Resonance (SPR) that the open channel has less affinity (measured as K_A) for FKBP12, than the closed channel. In this study the effect of phosphorylation on the binding of RyR1 to FKBP12 in the open or closed state was tested. Phosphorylation by PKA was measured by Western blot. SPR results showed that K_A, for FKBP12 of RyR1 in the closed states (EGTA) was significantly reduced by both phosphorylation and K201 to the values of the open state of the channel (1μM Ca^{2+}). This resulted from an increase in k_d, with the rate of association (k_a) remaining constant. In [^{3}H]Ryanodine binding assays, FKBP12 and K201individually decreased the open probability (P_o) of the channel by about 40% whether the channel was phosphorylated or not and there was no additive effect. Phosphorylation had no effect on the Ca^{2+} dose response curve whereas K201 significantly reduced the P_o. Both phosphorylation and K201 reversed the effects of Mg^{2+} inhibition. The results are explained through a model which proposes that both phosphorylation and K201 acted similarly to change the conformation of RyR1, but K201 stabilised the conformation whereas phosphorylation facilitated a subsequent molecular event that triggered channel opening.

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Muscle From Mice Heterozygous For A Mutation That Abolishes FKBP12 Binding To RyR1 Fatigue More Slowly Than Wildtype Muscle

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We created mice with a V2462D mutation in RyR1 to abolish FKBP12 binding. The soleus and diaphragm muscles of the V2462D heterozygous mice are more resistant to fatigue than the corresponding muscles from wildtype mice. There are, however, no differences in twitch force and maximal force at stimulation frequency range (15–300Hz) in the soleus and diaphragm between groups. Ca^{2+} transients from V2462D heterozygous myotubes show a smaller fractional decrease in the Ca^{2+} transient with repetitive stimulation than WT myotubes. Maximal voltage-gated Ca^{2+} release in V2462D heterozygous myotubes is smaller than that of WT myotubes. The homozygous V2462D mutation is late embryonic or birth lethal and myotubes from 18.5 day embryos show markedly reduced Ca^{2+} currents, voltage-gated Ca^{2+} transients and Ca^{2+} stores. Our data suggest that FKBP12 modulates the gain of E-C coupling in skeletal muscle and a small reduction in E-C coupling gain slows muscle fatigue. This work was supported by grants from NIH (AR41802) and MDA to SLH.

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S165F Mutation In Junctophilin-2 Affects Phosphorylation And Ca^{2+} Signaling In Skeletal Muscle

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Junctophilins (JPs) contribute to formation of triad junctions in muscle cells by physically linking the transverse-tubule and sarcoplasmic reticulum (SR) membranes. In human hypertrophic cardiomyopathy (HCM) patients, mutations in JP2 are linked to altered Ca^{2+} signaling in cardiomyocytes, whereas the effect of such mutations on skeletal muscle function has not been examined. Here we identify a dominant-negative role of a JP2 mutant associated with HCM (S165F) on ryanodine receptor (RyR1)-mediated Ca^{2+} release in skeletal muscle. Over-expression S165F in skeletal myotubes causes reduction of both KCl- and caffeine-induced Ca^{2+} release from SR, without altering the total intracellular Ca^{2+} storage. Consistent with the hypertrophic phenomenon observed with cardiac muscle, expression of S165F in skeletal muscle leads to significant increase in myotube diameter. Immunoprecipitation with anti-phosphoserine antibody reveals defective phosphorylation of S165F compared with the wild-type JP2. Since skeletal muscle express abundant amount of endogenous JP2, S165F-overexpression mediated reduction in excitation-contraction coupling machinery in skeletal muscle suggests a dominant-negative effect of the S165F mutant over the native JP2 on intracellular Ca^{2+} signaling, which may be linked to altered phosphorylation of JP2 or altered cross-talk between transverse-tubule and SR membranes.

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Independent Actions Of Junctin And Triadin On Skeletal Muscle RyR1 Channels

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Two similar proteins, junctin and triadin, are associated with the membrane of the sarcoplasmic reticulum (SR) Ca^{2+} store in skeletal muscle and have been thought to serve a common function in anchoring the Ca^{2+} binding protein calsequestrin (CSQ1) to the type 1 ryanodine receptor (RyR1). The resulting CSQ1/junctin/triadin/RyR1 complex is thought to mediate a functional response in the ion channel which conserves the Ca^{2+} stored within the SR. The individual actions of triadin and junctin on RyR1 and their contribution to the functional interaction between CSQ1 and RyR1 have not previously been examined and are reported here. Highly purified triadin or junctin added to the luminal side of purified RyR1 channels in lipid bilayers caused an increase in channel open time and stabilized channel openings to the maximum conductance. Competition studies indicated that triadin and junctin exert independent actions on RyR1. In addition, purified CSQ1 inhibited junctin/triadin-associated or junctin-associated, but not triadin-associated, RyR1 channels in the presence of 1mM luminal Ca^{2+}. As with native RyR1, purified RyR1 channels associated with either CSQ1/triadin/junctin, or CSQ1/junctin were further inhibited when luminal [Ca^{2+}] was reduced from 1mM to $\leq$100μM, in the same way as the native RyR1 channel complex. In marked contrast, the channel activity of the CSQ1/triadin/RyR1 complex increased when luminal Ca^{2+} was lowered, in a similar manner to the un-associated purified RyR1. These results indicate that junctin alone is responsible for mediating signals between luminal Ca^{2+}, CSQ1 and RyR1 and that triadin does not contribute to this function. Other evidence indicates that triadin plays an independent role in supporting Ca^{2+} release during excitation-contraction coupling (Goonasekara et al. 2007 *J. gen. Physiol*. 130, 365; Wang et al. 2008 *Cell Calcium* in press).